CLAIMS

What is claimed is:

1. A eukaryotic cell, comprising:
(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;
(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;
(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and
(d) a fourth nucleotide sequence encoding at least two different viral glycoproteins.

2. The cell of claim 1, wherein said cell further comprises a fifth nucleotide sequence having a 5' and a 3' end, said fifth nucleotide sequence encoding a desired protein, said fifth nucleotide sequence operably linked at said 5' end to a first retroviral long terminal repeat sequence and operably linked at said 3' end to a second retroviral long terminal repeat sequence.

3. The cell of claim 2, wherein said desired protein is a marker.

4. The cell of claim 3, wherein said marker is a fluorescent protein.

5. The cell of claim 1, wherein said two different viral glycoproteins are togaviral glycoproteins.

6. The cell of claim 5, wherein said togaviral glycoproteins are alphaviral glycoproteins.

7. The cell of claim 6, wherein said alphaviral glycoprotein is a Ross River alphaviral glycoprotein.

8. The cell of claim 1, wherein said eukaryotic cell is a mammalian cell.

9. The cell of claim 8, whererin said mammalian cell is a human cell.

10. The cell of claim 1, wherein said retroviral Gag, Pol and Pro polypeptides are comprised of Moloney murine leukemia Gag, Pro and Pol polypeptides.

11. The cell of claim 1, wherein said cell produces a pseudotyped retrovirus having a lipid bilayer, said viral glycoproteins disposed in said lipid bilayer.

12. The cell of claim 1, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

13. A eukaryotic cell, comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding a filoviral glycoprotein, said first, second, third and fourth nucleotide sequences being chromosomally-integrated, said cell stably producing pseudotyped retroviruses.

14. The cell of claim 13, wherein said cell further comprises a fifth nucleotide sequence having a 5' end and a 3' end, said fifth nucleotide sequence encoding a desired protein, said fifth nucleotide sequence operably linked at said 5' end to a first retroviral long terminal repeat sequence and operably linked at said 3' end to a second retroviral long terminal repeat sequence.

15. The cell of claim 13, wherein said filoviral glycoprotein is selected from the group consisting of Marburg virus glycoprotein and Ebola virus glycoprotein.

16. The cell of claim 13, wherein said retroviral Gag, Pro and Pol polypeptides are comprised of Moloney murine leukemia virus Gag, Pro and Pol polypeptides.

17. The cell of claim 13, wherein said cell produces pseudotyped retrovirus at a titer of at least about 4.5×10^4 transforming units/ml of supernatant.

18. A eukaryotic cell, comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding a Marburg virus glycoprotein.

19. A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:

transfecting a eukaryotic cell with a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide, a third nucleotide sequence encoding a retroviral Pol polypeptide and a fourth nucleotide sequence encoding at least two different viral glycoproteins.

20. The method of claim 19, wherein said first, second and third nucleotide sequences are operably linked to a promoter sequence.

21. The method of claim 19, wherein said viral glycoproteins are togaviral glycoproteins.

22. The method of claim 21, wherein said togaviral glycoproteins are alphaviral glycoproteins.

23. The method of claim 22, wherein said alphaviral glycoproteins are Ross River alphaviral glycoproteins.

24. The method of claim 19, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

25. The method of claim 19, wherein said cell further comprises a fifth nucleotide sequence having a 5' end and a 3' end, said fifth nucleotide sequence encoding a desired protein, said fifth nucleotide sequence operably linked at said 5' end to a first retroviral long terminal repeat sequence and operably linked at said 3' end to a second retroviral long terminal repeat sequence.

26. A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:

(a) transfecting a eukaryotic cell with a vector including a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide and a third nucleotide sequence encoding a retroviral Pol polypeptide, said first, second and third nucleotide sequences operably linked to a first promoter sequence; and

(b) transfecting said cell with a fourth nucleotide sequence encoding at least two viral glycoproteins, said fourth nucleotide sequence operably linked to a second promoter sequence.

27. The method of claim 26, said method further comprising transfecting said cell with a vector including a fifth nucleotide sequence having a 5' and a 3' end, said fifth nucleotide sequence encoding a desired protein, said fifth nucleotide sequence operably linked at said 5' end to a first retroviral long terminal repeat sequence and operably linked at said 3' end to a second retroviral long terminal repeat sequence.

28. The method of claim 26, wherein said desired protein is a marker.

29. The method of claim 26, wherein said first, second, third and fourth nucleotide sequences are chromosomally-integrated.

30. A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:

(a) transfecting a eukaryotic cell with a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide, a third nucleotide sequence encoding a retroviral Pol polypeptide and a fourth nucleotide sequence encoding a

filoviral glycoprotein, said first, second, third and fourth nucleotide sequences being chromosomally-integrated, said cell stably producing pseudotyped retroviruses.

31. The method of claim 30, wherein said filoviral glycoprotein is selected from the group consisting of Ebola virus glycoprotein and Marburg virus glycoprotein.

32. A method of forming a eukaryotic cell for producing pseudotyped retroviruses, said method comprising:

transfecting a eukaryotic cell with a first nucleotide sequence encoding a retroviral Gag polypeptide, a second nucleotide sequence encoding a retroviral Pro polypeptide, a third nucleotide sequence encoding a retroviral Pol polypeptide and a fourth nucleotide sequence encoding a Marburg virus glycoprotein.

33. A pseudotyped retrovirus, comprising:

(a) a retroviral capsid;

(b) a lipid bilayer; said lipid bilayer surrounding said retroviral capsid; and

(c) at least two different viral glycoproteins disposed in said lipid bilayer.

34. The retrovirus of claim 33, said retrovirus further comprising a nucleotide sequence encoding a desired protein, said nucleotide sequence enclosed within said retroviral capsid.

35. The retrovirus of claim 33, wherein said viral glycoproteins are togaviral glycoproteins.

36. The retrovirus of claim 35, wherein said togaviral glycoproteins are alphaviral glycoproteins.

37. The retrovirus of claim 36, wherein said alphaviral glycoproteins are Ross River alphaviral glycoproteins.

38. The retrovirus of claim 33, wherein said retroviral capsid is comprised of a Moloney murine leukemia virus capsid.

39. A pseudotyped retrovirus, comprising:

(a) a retroviral capsid;

(b) a lipid bilayer; said lipid bilayer surrounding said retroviral capsid; and

(c) a Marburg virus glycoprotein disposed in said lipid bilayer.

40. A method of introducing a nucleotide sequence into a cell, said method comprising:

transducing a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with a pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer; said lipid bilayer surrounding said retroviral capsid;

at least two different viral glycoproteins disposed in said lipid bilayer; and

a desired ribonucleotide sequence.

41. The method of claim 40, wherein said retroviral capsid is a Moloney murine leukemia virus capsid.

42. The method of claim 40, wherein said virus having at least two different glycoproteins in its lipid bilayer is a togavirus, and said at least two different viral glycoproteins are togaviral glycoproteins.

43. The method of claim 42, wherein said togavirus is an alphavirus and said togaviral glycoproteins are alphaviral glycoproteins.

44. A method of introducing a nucleotide sequence into a cell, said method comprising:

transducing a cell permissive for Marburg virus entry with a pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer; said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer; and

a desired ribonucleotide sequence.

45. A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a pseudotyped retrovirus with said agent, said pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

at least two different viral glycoproteins disposed in said lipid bilayer; and

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid;

(b) treating a cell permissive for entry of a virus having at least two different viral glycoproteins disposed in its lipid bilayer with said treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker.

46. The method of claim 45, wherein said virus having at least two different viral glycoproteins disposed in its lipid bilayer is a togavirus and said two different viral glycoproteins are togaviral glycoproteins.

47. The method of claim 46, wherein said togavirus is an alphavirus and said togaviral glycoproteins are alphaviral glycoproteins.

48. The method of claim 45, wherein said agent is an immunological agent.

49. The method of claim 45, wherein said agent is a pharmacological agent.

50. A method of screening agents effective in blocking Marburg virus entry into a cell, said method comprising:

(a) treating a pseudotyped retrovirus with said agent, said pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer; and

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid;

(b) treating a cell permissive for Marburg virus entry with said treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker.

51. A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with said agent;

(b) contacting said treated cell with a pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

at least two different viral glycoproteins disposed in said lipid bilayer;

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker.

52. A method of screening agents effective in blocking viral entry into a cell, said method comprising:

(a) treating a cell permissive for entry of a Marburg virus with said agent;

(b) contacting said treated cell with a pseudotyped retrovirus having

a retroviral capsid;

a lipid bilayer, said lipid bilayer surrounding said retroviral capsid;

a Marburg virus glycoprotein disposed in said lipid bilayer;

a nucleotide sequence encoding a desired marker, said nucleotide sequence enclosed within said retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker.

53. A kit for forming a pseudotyped retrovirus, said kit comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding at least two different viral glycoproteins.

54. The method of claim 52, wherein said viral glycoproteins are togaviral glycoproteins.

55. A kit for forming a pseudotyped retrovirus, said kit comprising:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding a Marburg virus glycoprotein.